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UTILIZATION OF PHOSPHORUS FROM APATITE AND GROWTH OF PLANTS INOCULATED WITH VESICULAR ARBUSCULAR MYCORRHIZA AND PHOSPHATE DISSOLVING BACTERIA

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ABSTRACT

An annual grass, *Pennisetum ploystechyon (L) Schult* and a perennial creeping legume *Pueraria phaseoloides* took up more P and grew better in an acid soil (pH 4.8) treated with a poorly soluble source of apatite. Inoculation of the sterilized soil with a heterogenous mixture of vesicular arbuscular mycorrhizal (VAM) spores extractedfrom the soil and with *Glomus fasciculatus* (E3) spores did not increase plant growth or uptake of P in soil *with* apatite but growth was increased considerably in soil without apatite. However, inoculation with E3 type spores had no effect on growth or P uptake of *Pennisetum*. Phosphate dissolving bacteria (PDB) stimulated growth of *Pueraria* and uptake of P from a soil enriched with apatite.

Key Words: Apatite, *Glomus fasciculatus, Pennisetum polystechyon, phosphate* dissolving bacteria, *Pueraria phaseoloides.*

INTRODUCTION

Plants infected with vesicular arbuscular mycorrhiza (VAM) are known to be more effective in the uptake of P from rock phosphates and soils low in available P than are uninfected plants (Mosse 1973 a). There is evidence that P from poorly -soluble rock phosphate may only be available to mycorrhizal plants (Powell and Deniel 1978). This is attributed to an increased effective surface area of absorption in VAM which compensates for the low mobility of P in soil (Tinker 1975).

Phosphate dissolving bacteria (PDB) in the rhizosphere are known to dissolve rock phosphates (Swaby and Sperber 1959) leading to improved P uptake in plants (Azcon *et al.* 1976). VAM and PDB possibly act synergistically to improve growth and P nutrition of plants (Barea *et al* 1975).

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We have investigated the role of VAM and PDB in the uptake of P using a low citric acid-soluble apatitic rock phosphate.

MATERIALS AND METHODS

Apatite

Finely ground Eppawala (Sri Lanka) apatite (212μm) containing 12.0% P mainly in the form of chloroapatite and francolite (Anon 1980) was used. It's citric acid-soluble P content was 1.58% and pH in water was 7.59.

Soil

The top Layer (0 - 20cm, A horizon) of a red yellow podzolic soil from the Rubber Research Institute (Dartonfield Estate) having a pH of 4.8 (1:2.5 in water) and available P content of 5.9 $\mu g. g.^{-1}$ as determined by Bray's method (Dickman and Bray 1940) was used.

Sterilization of Soil and Pots

In Experiment 1, soil passing through a 1.0 cm. mesh sieve was heaped into mounds of 180 x 120 x 30cm, covered with black polythene sheeting, and fumigated twice during 2 weeks, each time with 500g. of a commercial fumigant containing 98% methyl bromide and 2% chloropicrin. Six kg of soil was placed in each pot which had previously been surface sterilized with 2% formaldehyde. In Experiment 2, soil was sterilized by autoclaving (2 h at 120°C) on two occasions with a 2 day interval between them. Soil (1kg each) was transferred into pots sterilized with 2% formaldehyde. In both experiments seed were sown 2 weeks after soil sterilization as plant establishment was poor when seed were sown immediately after sterilization.

Basal fertilizers

Soil in each pot of Experiment 1 received 2g urea, 1g KCl and 0.5g $ZnSo_4$, at the begining of the experiment, and after the first harvest of the shoots. In Experiment 2 the same rates of fertilizer were added to soil in each pot before sowing.

Mycorrhizal inocula

Two types of VAM inocula and a control were used. One type consisted of spores of *Glomus* species (Jayaratna 1982) from Dartonfield soil where *Pueraria* was growing as ground cover under *Hevea* (rubber). A few spores

(15%) of *Acaulospora* and *Gigaspora* species (8%) were also present. The second inoculum comprised spores of strain E3 *(Glomus fasciculatus)* obtained from sand culture pots of *Pueraria* maintained in a greenhouse. To collect spores, soil from culture pots were wet sieved and decanted (Gerdeman and Nicolson 1963).

Concentrated sulphuric acid-treated *Pueraria* seeds (50 per tray) or hypochlorite surface-sterilized *Pennisetum* seeds (200 per tray) were sown in shallow aluminium trays (50 x 40 cm) containing a 1:1 mixture of sterilized soil sand mixture. Each tray was inoculated with a VAM inoculum comprising about 500 spores in 50 ml suspension of water. Plants for the non-mycorrhizal treatments were also raised in similar trays without VAM inoculum. All trays received a filtrate of the decanted unsterilized soil from the field to ensure the presence of other microorganisms in the medium.

The VAM inoculum for Experiment 2 was the same as the field inoculum of Experiment 1, and the spores were surface sterilized with 0.5% sodium hypochlorite (Sward 1981). About 300 of these spores were added to each pot (15 cm diam). containing sterile soil-sand mixture in which 90 seeds were planted for inoculation. Plants for the non-mycorrhizal treatments were also raised in similar pots. All plants were allowed to grow for 2 weeks before inoculation. A soil filtrate as described in Experiment 1 was added to all pots.

Bacterial inocula

Phosphate-dissolving bacteria (PDB) for Experiment 2 were isolated from the rhizosphere of *Pueraria* growing under *Hevea*. The medium contained soil extract, 1.0% glucose and precipitated apatite (Katznelson *et al*, 1962). Plants inoculated with VAM were inoculated with PDB by immersing their roots in a culture suspension containing 10^8 cells ml^{-1}. All bacterial colonies other than PDB growing in the soil extract medium were collected, reisolated in the same medium and suspended to give 10^8 cells ml^{-1}: 50ml of this suspension were added to all pots to ensure the presence of other soil bacteria in the growth medium.

Estimation of root length

Total length of roots were estimated using the grid line intersect method (Newman, 1966: Marsh, 1971).

Percentage mycorrhizal infection

Root samples were cleared and stained (Phillips and Hayman, 1970) and 25 of these 1.0 cm root pieces were observed under a stereo microscope and scored for percentage infection on a relative scale.

Experimental conditions, designs and treatments

Experiment 1 had a 2 x 3 x 2 factorial design and investigated the effect of apatite (O and 24 mg P kg $^{-1}$ soil) and inoculation with E3 or heterogeneous inoculum of VAM or no inoculum on growth an P uptake of *Pennisetum* and *Pueraria*.

In Experiment 2 the effect of apatite (O and 24 mg P kg^{-1} soil), a heterogenous VAM inoculum as in Experiment 1, and PDB on growth and P uptake in *Pueraria* were investigated in a 2 x 2 x 2 factorial design.

In both experiments each treatment was replicated three times and pots were arranged in a randomized block design in a greenhouse where the mean day and night temperatures during the Experiment were 30 ± 2°C and 23 ± 2°C . The pots were watered daily to field capacity.

Shoots were harvested twice at 2 monthly intervals in the case of Experiment 1 and at monthly intervals in Experiment 2, and DM yields recorded. Roots were harvested at the end of the experiments.

P content in shoots was determined in Se - H_2SO_4 digested samples by automated colorimetric method using vanadomolybdate (Singh and Ratnasingham 1971).

RESULTS

Experiment 1

Analysis of variance revealed that a crop x mycorrhiza interaction was significant for shoot DM, shoot P and root length. A crop x apatite interaction was evident only for shoot DM and an apatite x mycorrhiza interaction was not significant for any of the three variables measured.

Shoot dry metter yield was significantly (P=0.001) higher when *Pennisetum* was grown in soil with added apatite than in soil without (Table 1). However with *Pueraria* the difference although large failed to reach significance (P=0.001) but mycorrhizal infection was less in apatite-treated soil than the untreated.

Inoculation with either the field VAM inoculum or E3 inoculum highly (P=0.01). stimulated shoot and root growth of *Pueraria*: the same was true for *Pennisetum* only with the field inoculum. This is consistent with the percentages of infection recorded (Table 2). Some VAM contamination was observed in the uninoculated controls of both species which however did not mask the VAM inoculant effects.

Although the apatite x VAM interaction was not significant there are indications that the magnitude of response for the three variables measured to VAM inoculation is greater in the absence of apatite than in its presence (Table 3).

Experiment 2.

Analysis of variance showed that unlike in Experiment 1 the apatite x VAM interaction was significant here, so was apatite x PDB, but not VAM x PDB.

Apatite greatly improved both shoot and root growth of non-mycorrhizal but not of mycorrhizal *Pueraria* (Table 4). A similar trend was also evident with the shoot P yields although the interaction here was not significant possibly because of the high (53%) coefficient of variation in the data. There is also strong indication that apatite stimulated multiplication of PDB which was further enhanced when VAM was also present.

PDB greatly stimulated shoot and root growth and shoot P yield in the presence of apatite (Table 5). However, the inhibitory effect of apatite on all variables measured when PDB was not present is inexplicable.

DISCUSSION

Response to apatite was similar for both *Pennisetum* and *Pueraria* in Experiment 1 (Table 1) except that DM yield of *Pueraria* with apatite was not significantly higher than without it. Apatite increased available soil P and thereby enhanced root development, which more than compensated for its partial inhibition of VAM infection, in terms of increased shoot DM and P yield. Improved root development (Tinker, 1975, Newman and Andrews 1973) and decreased VAM infection (Mosse 1973 B) are common features of increased available P in the soil.

Of the two types of VAM inocula (Table 2) both the field inoculum and E3 were equally effective with *Pueraria,* but E3 was not effective with *Pennisetum* although some infection was observed. This is to be expected as roots of grasses usually form effective VAM association with fine hyphae mycorrhiza and the unspecified inoculum possibly contained such mycorrhiza.

The apatite x VAM interaction surprisingly failed to be significant in Experiment 1 (Table 3) possibly due to the very high coefficient of variation. But the indications are strong that mycorrhizal effect, as was to be expected, was grater for all variables when apatite was absent.

Far more than in Experiment 1, (Table 3) mycorrhizal *Pueraria* in Experiment 2 (Table 4) greatly stimulated plant growth only in unamended soil and not in soil amended with apatite. It implies that amendment of soil with apatite (24 mg P kg^{-1}) increased the available soil P to an extent that negated the benefit of VAM in terms of uptake. In other words at the available soil P level (5.9 mg g^{-1}), *Pueraria* if infected with effective VAM may not be limited by P for growth in that environment.

There is evidence that inoculation with PDB stimulated plant growth and P uptake when phosphate was added. Corresponding increases in the number of PDB (Table 4) substantiate this observation. Apart from solubilizing low soluble phosphates and making them available to the host plants, PDB are also known to stimulate plant growth through secretion of growth substances which the roots absorb (Brown 1974). Such substances can also enhance root development and hence phosphate uptake thus producing a further indirect effect (Bowen and Rovira 1961). A synergistic effect had been reported (Barea *et al* 1975) when both PDB and VAM are present as against one or none, but there is no such evidence in data.

The depression of growth in apatite-treated plants in the absence of PDB is hard to explain. It seems possible that PDB counteracted some adverse effect of apatite or this result may be an artifact.

Table 1. Effect of apatite on shoot DM yield P content, root length and percentage VAM infection of *Pueraria* and *Pennisetum*

	Shoot Dm (g pot^{-1})		Shoot P content (mg pot^{-1})		Root length (m pot^{-1})		% VAM infection	
	−apatite	+apatite	−apatite	+apatite	−apatite	+apatite	−apatite	+apatite
Pueraria	5.82	9.18	9.42	18.37	20.3	39.7	31.2	22.6
Peninsetum	9.53	20.09	8.64	20.34	92.8	133.2	20.8	14.3
LSD P=0.001) (Species x apatite) Interaction)	3.53		NS		NS		Not analysed	
Apatite means for both species	—		9.11		18.36*	56.6	86.5*	

* Difference significant (P=0.001)

NS = Not significant

Table 2. Effect of VAM inoculation on shoot Dm yield, P content, root length and percentage VAM infection.

	Shoot DM (g pot^{-1})			Shoot P content (mg pot^{-1})			Root length (m pot^{-1})			% VAM infection		
	NM	FM	E_3	NM	FM	E_3	NM	FM	E_3	NM	FM	E_3
Pueraria	1.68	9.98	10.83	2.69	17.23	18.63	5.3	44.6	40.0	2.8	39.0	39.0
Pennisetum	8.02	27.72	7.72	8.04	29.44	6.33	92.4	167.9	78.9	4.5	35.8	12.3
LSD (P–0.01)												
(Species x mycorrhiza interaction)		4.32			5.33			33.7			Not analysed	

NM=No VAM inoculum, FM=Field inoculum, E_3 *Glomus fasciculatus*

Table 3. Effect of VAM inoculation on shoot DM yield, P content, root length and percentage root infection for both plant species.

	Shoot DM (g pot-^{1})			Shoot P content (mg pot-^{1})			Root length (m pot-^{1})			% VAM infection		
	NM	FM	E_3	NM	FM	E_3	NM	FM	E_3	NM	FM	E_3
Apatite	8.1	24·2	11.6	9.10	30.28	15.69	70.9	131.4	57.3	6.5	30.0	19.0
No apatite	1.6	15.0	6.5	1.63	16.05	9.43	26.7	81.7	61.7	1.0	44.8	19.0
Interaction		NS			NS			NS			Not analysed	

NM = No VAM inoculum FM = Field inoculum E3 = *Glomus fasciculatus*

NS = Not significant

Table 4. Effect of apatite and VAM on shoot Dm yield, P content and root length of *Pueraria* and count of PDB.

	Shoot Dm (g pot-^{1})		Shoot P content (mg pot-^{1})		Root length (m pot-^{1})		Count of PDB (x166 g-^{1} dry soil)	
	–apatite	+apatite	–apatite	+apatite	–apatite	+apatite	–apatite	+apatite
– VAM	0.60	2.04	0.41	1.38	24.6	60.7	9.5	20.0
+VAM	2.24	2.17	1.62	1.58	73.1	61.1	7.0	32.5
LDS (P=0.001)								
(VAM x apatite interaction)		0.57		NS		18.3		Not analysed

NS = Not significant

Table 5. Effect of apatite and PDB on shoot DM yield, P content, root length and percentage VAM infection of *Pueraria*.

	Shoot DM (g pot^{-1})		Shoot P content (mg pot^{-1})		Root length (m pot^{-1})		% VAM infection	
	−apatite	+apatite	−apatite	+apatite	−apatite	+apatite	−apatite	+apatite
-PDB	1.52	0.58	0.96	0.40	56.6	28.1	38	50
+PDB	1.35	3.63	1.07	2.50	41.2	93.8	43	41
LSD (P=0.01)								
(Apatite x PDB interaction)	0.56		0.82		18.2		Not analysed	

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